

CLAIMS

What is claimed is:

- Sub B1
- 10084501-022802
- Sub B1
1. A transgenic animal whose genome contains a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes wherein said animal develops cancer.
 2. A cell from the transgenic animal of claim 1.
 3. The transgenic animal of claim 1 wherein the animal is a mouse.
 4. A cell from the transgenic animal of claim 3.
 5. A cell of claim 4 which is selected from the group consisting of stem cells, epithelial cells and myelofibroblasts.
 6. The transgenic mouse of claim 3 wherein the genetic background of the mouse is selected from the group consisting of a B6 mouse, a 129Sv/J hybrid mouse, a 129S3 hybrid mouse and a $\frac{1}{2}$ B6, $\frac{1}{4}$ 129Sv/J and $\frac{1}{4}$ 129S3 hybrid mouse.
 7. A transgenic mouse as in claim 3 which further comprises a mouse which is a germ free mouse.
 8. A transgenic animal as in claim 3 wherein the cancer is selected from the group consisting of ileal cancer and myeloleukemia.
 9. An animal model for cancer which comprises a transgenic animal whose genome comprises a homozygous disruption of the endogenous *Gpx1* gene and a homozygous disruption of the endogenous *Gpx2* gene and wherein disruption of the *Gpx1* and *Gpx2* genes is sufficient to effect one or more signs or symptoms in the animal associated with cancer.
 10. The model of claim 9 wherein the transgenic animal is a mouse.
- 5
- Sub A1

Sub B1
11. The model of claim 10 wherein the cancer is ileal cancer.

12. The model of claim 11 wherein the sign or symptom associated with cancer is selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, dysplasia in the small bowel, one or more tumors in the small bowel.

Sub B1
13. The model of claim 10 wherein the cancer is myeloleukemia.

14. The model of claim 10 wherein the genetic background of the mouse is selected from the group consisting of a B6 mouse, a 129Sv/J hybrid mouse, a 129S3 hybrid mouse and a 1/2 B6, 1/4 129Sv/J and 1/4 129S3 hybrid mouse.

15. A model as in claim 10 wherein the mouse further comprises a mouse which is a germ free mouse.

16. A method to screen for potential therapeutic agents for the treatment of cancer which comprises the steps of:

a) administering a potential therapeutic agent to a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes and

b) maintaining the animal for a time sufficient to permit the detection a change in one or more signs or symptoms in the animal associated with cancer in the transgenic animal;

c) observing the animal for a change in at least one sign or symptom associated with cancer, wherein a second transgenic animal having the same genetic background as the first transgenic animal and whose genome also comprises a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes has been maintained under the same conditions as the first animal but has not received the potential therapeutic agent; and

d) determining whether one or more signs or symptoms associated with cancer is present in the second transgenic animal but not in the first transgenic animal;

wherein a potential therapeutic agent will be one that causes a lower incidence of at least one sign or symptom associated with cancer in the first transgenic animal.

17. A method as in claim 16 wherein the transgenic animal is a mouse.

18. A method as in claim 17 wherein the cancer is ileal cancer.

19. The model of claim 18 wherein the sign or symptom associated with cancer is selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perinatal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, dysplasia in the small bowel, one or more tumors in the small bowel.

20. The method of claim 17 wherein the cancer is myeloleukemia

21. A method as in claim 17 wherein the genetic background of the mouse is selected from the group consisting of a B6 mouse, a 129Sv/J hybrid mouse, a 129S3 hybrid mouse and a 1/2 B6, 1/4 129Sv/J and 1/4 129S3 hybrid mouse.

22. A method as in claim 17 wherein the mouse further comprises a mouse which is a germ free mouse.

23. A method as in claim 17 wherein determination of whether one or more signs or symptoms associated with cancer is present in the second mouse but not in the first mouse comprises sacrificing the first and second mouse after a time sufficient for the detection of at least one sign or symptom associated with cancer in the first mouse and second mouse has elapsed.

24. The method as in claim 17 wherein the determination of whether one or more signs or symptoms associated with cancer is present in the second mouse but not in the first mouse comprises withdrawing a body fluid or other bodily substance from the first and second mouse and analyzing the body fluid for the presence of one or more signs or symptoms associated with cancer is present.

25. The method as in claim 24 wherein the bodily fluid is selected from the group consisting of blood and stool.

26. A transgenic animal whose genome comprises a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes wherein the animal's genome additionally comprises a DNA sequence encoding a heterologous gene of interest.

27. A transgenic animal as in claim 26 which is a mouse.

28. The mouse of claim 27 wherein the heterologous gene of interest is selected from the group consisting of an antiangiogenic protein, an immunomodulator, a ribozyme, a peptide and an antisense nucleic acid.

29. A transgenic mouse as in claim 29 wherein the mouse is a mouse that is selected from the group consisting of a B6 mouse, a C57Bl6/J hybrid, a 129Sv/J hybrid, a 129S3 hybrid and 1/2 B6, 1/4 129SvJ and 1/4 129S3 hybrid.

30. A transgenic mouse as in claim 29 wherein the hybrid mouse is 1/2 B6, 1/4 129SvJ and 1/4 129S3.

31. A method for assessing the therapeutic effect of a heterologous gene of interest on the development of cancer which comprises the steps of:

a) expressing the heterologous gene of interest in a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes, and

b) maintaining the first transgenic animal for a time sufficient to permit the detection a change in one or more signs or symptoms in the first transgenic animal associated with cancer in the first transgenic animal;

c) observing the first transgenic animal for a change in at least one sign or symptom associated with cancer, wherein a second transgenic animal having the same genetic background as the first transgenic animal and comprising a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes does not express the gene

of interest, wherein the second transgenic animal has been maintained under the same conditions as the first transgenic animal; and

d) determining whether one or more signs or symptoms associated with cancer is present in either the second animal, wherein a gene of interest which reduces cancer will be one that causes a lower incidence of at least one sign or symptom associated with cancer in the first animal.

32. A method as in claim 31 wherein the transgenic animal is a mouse.

33. A method as in claim 32 wherein the mouse is a mouse that is selected from the group consisting of a B6 mouse, a C57Bl6/J hybrid, a 129Sv/J hybrid, a 129S3 hybrid and 1/2 B6, 1/4 129SvJ and 1/4 129S3 hybrid.

34. A transgenic mouse as in claim 33 wherein the hybrid mouse is 1/2 B6, 1/4 129SvJ and 1/4 129S3.

35. A method of identifying markers associated with cancer, the method comprising: comparing the presence, absence or level of expression of at least one gene or protein in a transgenic animal whose genome comprises a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes with the level or expression of the gene or protein in a second animal, wherein the second animal has the same genetic background as the first animal but does not comprise a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes, wherein the difference between the transgenic animal and the second animal in the presence, absence or level of expression of the gene or protein indicates that the expression of the gene is a marker associated with cancer.

36. A method as in claim 34 wherein the transgenic animal is a mouse.

37. A method as in claim 36 wherein the mouse is a mouse that is selected from the group consisting of a B6 mouse, a C57Bl6/J hybrid, a 129Sv/J hybrid, a 129S3 hybrid and 1/2 B6, 1/4 129SvJ and 1/4 129S3 hybrid.

38. A transgenic mouse as in claim 37 wherein the hybrid mouse is $\frac{1}{2}$ B6, $\frac{1}{4}$ 129SvJ and $\frac{1}{4}$ 129S3.

39. A method as in claim 35 wherein the gene or protein is selected from the group consisting of telomerase and mucin antigen.

40. A transgenic double knockout mouse whose genome comprises a homozygous disruption of the endogenous *Gpx1* gene and a homozygous disruption of the endogenous *Gpx2* gene, wherein each disruption comprises the insertion of a transgene, and wherein the combined disruptions result in a decreased level of GPX-1 and GPX-GI production and decreased number of cells producing GPX-I and GPX-GI in the transgenic mouse as compared to a nontransgenic mouse.

41. A transgenic double knockout mouse as in claim 40 which exhibits one or more physiological symptoms selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease and cancer of the lower gastro-intestinal tract.

42. A cell isolated from a double knockout mouse as in claim 40.

43. A cell as in claim 42, selected from the group consisting of a stem cell, an epithelial cell and a myofibroblast.

44. A cell as in claim 43 which is a stem cell.

45. A cell as in claim 43 which is an epithelial cell.

46. A cell as in claim 43 which is a myofibroblast.

47. A transgenic double knockout mouse as in claim 40 which further comprises a mouse which is a germ free mouse.

48. A transgenic double knockout mouse as in claim 1 wherein said knockout mouse is a mouse with a B6 genetic background.

49. A transgenic double knockout mouse as in claim 1 wherein said knockout mouse is a mouse with a hybrid mouse having a $\frac{1}{2}$ B6, $\frac{1}{4}$ 129 SuJ and $\frac{1}{4}$ 129 S3 genetic background.

50. A method of selecting an agent for treating a metabolic disorder comprising:
(a) measuring at least one symptom selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease and cancer of the lower gastro-intestinal tract in a knockout mouse whose genome has been manipulated to comprise a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes, wherein the disruption of both the *Gpx1* gene and *Gpx2* genes results in said knockout mouse exhibiting one or more of said diseases, symptom or symptoms;

(b) administering an agent to said mouse;

(c) measuring one or more of said symptoms in the mouse after administering the agent; and

(d) comparing at least one of said disease, symptom or symptoms in the mouse before and after administering the agent, wherein a decrease in at least one of said diseases, symptom or symptoms after administering the agent indicates the agent is an agent for treating said disease, symptom or symptoms.

51. A method as in claim 50 wherein said knockout mouse is a mouse with a B6 genetic background.

52. A method of selecting an agent that modulates GPX enzyme activity comprising:
(a) administering an agent to a first group of isolated mouse cells and not to a second group of mouse cells, wherein the genomes of both the first and second isolated mouse cell groups have been manipulated to comprise a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes, and wherein the disruption of both the *Gpx1* gene and *Gpx2* genes prevents expression of functional GPX-1 and GPX-GI proteins; and

(b) determining the amount of GPX enzyme activity of the first and second cell groups, wherein a difference in the amount of proliferation of the first cell group as compared to the second cell group indicates that the agent modulates GPX enzyme activity.

53. The method of claim 52 wherein the mouse cells are selected from the group of cell types consisting of stem cells, epithelial cells, intestinal epithelial cells and myofibroblast cells.

54. The method of claim 53 wherein the mouse cells are epithelial cells.

55. The method of claim 54 wherein the epithelial cells are intestinal epithelial cells.

56. The method of claim 53 wherein the cells are stem cells.

57. The method of claim 53 wherein the cells are myofibroblasts.

58. A method of selecting an agent for treating a metabolic disorder comprising:

(a) measuring at least one symptom in a first double knockout mouse having a first genetic background, whose genome is manipulated to comprise a homozygous disruption of both the endogenous *Gpx1* and *Gpx2* genes, wherein the disruption of both the *Gpx1* and *Gpx2* genes results in said knockout mouse exhibiting a disease, symptom or symptoms selected from the group consisting of: ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease and cancer of the lower gastro-intestinal tract;

(b) measuring said symptom in a second double knockout mouse having a second genetic background, whose genome is manipulated to comprise a homozygous disruption of both the endogenous *Gpx1* and *Gpx2* genes, wherein the disruption of both the *Gpx1* and *Gpx2* genes results in said knockout mouse exhibiting at least one of said disease, symptom or symptoms;

(c) administering an agent to said first and second mouse;

(d) measuring one or more of said symptoms in the first and second mouse after administering the agent; and

(e) comparing at least one of said symptoms in said first and second mouse before and after administering the agent, wherein a decrease in said disease, symptom or symptoms after administering the agent indicates the agent is an agent for treating said disease, symptom or symptoms associated with a metabolic disorder.

59. The method of claim 57 wherein one of said first and second mouse has a B6 genetic background.

60. A transgenic mouse which has a homozygous knockout of the *Gpx1* gene and a heterozygous knockout of one allele of the *Gpx2* gene.

61. An animal model for the study of the degree of functional redundancy of GPX-1 and GPX-GI in the ileum and colon comprising the mouse of claim 60.

62. A transgenic mouse which has a homozygous knockout of the *Gpx2* gene and a heterozygous knockout of one allele of the *Gpx1* gene.

63. An animal model for the study of the degree of functional redundancy of GPX-1 and GPX-GI in the ileum and colon comprising the mouse of claim 62.

64. Isolated mammalian cells comprising a diploid genome including chromosomally incorporated transgenes, wherein the transgenes disrupt both alleles of the genomic *Gpx1* gene and *Gpx2* genes and inhibit expression of said genes.

65. The method of claim 57 wherein one of said first and second mouse has a 1/2 B6, 1/4 129 SvJ and 1/4 129 S3 background.

66. A method of selecting an agent for treating a metabolic disorder comprising:
(a) measuring at least one symptom selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease and cancer of the lower gastro-intestinal tract in a knockout cell whose genome has been manipulated to comprise a homozygous disruption of both the endogenous *Gpx1* gene and *Gpx2* genes, wherein the disruption of both the

Gpx1 gene and *Gpx2* genes results in said knockout mouse exhibiting one or more of said diseases, symptom or symptoms;

(b) administering an agent to said cell;

(c) measuring one or more of said symptoms in the cell after administering the agent; and

(d) comparing at least one of said disease, symptom or symptoms in the cell before and after administering the agent, wherein a decrease in at least one of said diseases, symptom or symptoms after administering the agent indicates the agent is an agent for treating said disease, symptom or symptoms.

208220.1294800T